

Differences in Variation in Egg Size for Several Species of Salamanders (Amphibia: Caudata) That Use Different Larval Environments

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ABSTRACT—Comparative descriptive data are provided on variation of egg size in five species of salamanders. The species differ in their use of larval habitats. *Ambystoma maculatum* uses temporary, rain-filled pools in the southern Appalachian Mountains. *Desmognathus aeneus* is a direct developer and is not constrained by risk of larval desiccation. The remaining three species, *Eurycea wilderae*, *D. ochrophaeus*, and *D. santeetlah*, have permanent streams as their larval environment. Using the coefficient of variation (CV), I document both variation within individual clutches and variation at the interclutch level. The degree of variation differs among individual clutches and among species. Variation at the intraclutch level does not agree with that predicted. However, variation at the interclutch level conforms to the prediction that *A. maculatum* (which utilizes ephemeral larval environments) exhibits the highest degree of variation in egg size.

In many populations of biphasic amphibians, the key factor underlying the timing of metamorphosis and larval survivorship is the time for which the larval environment remains hospitable. In a permanent larval habitat, where mortality from desiccation is unlikely, the larval period of an amphibian can be long. For example, paedomorphic species of salamanders inhabit permanent bodies of water. However, many species of amphibians inhabit larval environments that are temporary and unpredictable, and desiccation is a threat to species that use those bodies of water. Because environmental pressure to escape the larval environment can vary from year to year, species that breed in temporary pools may exhibit different reproductive strategies than species that use permanent bodies of water.

Parental investment, one facet of the study of reproductive strategies, has been the subject of theoretical and/or empirical studies in amphibians (e.g., Wilbur 1977; Kaplan 1980, 1985; Crump 1981, 1984; Kaplan and Cooper 1984). These studies have documented the

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extensive variation in propagule size in amphibians. Such variation has been interpreted as an "evolved tactic" (Capinera 1979) that ensures that viable offspring are produced in variable habitats. In many species of amphibians with complex life cycles, vitellogenesis occurs in the terrestrial habitat. The female then may not be able to receive environmental cues that indicate the size of eggs she should produce to ensure survival of offspring in the aquatic habitat. Because offspring can be exposed to a habitat that is variable, there should be an optimal range in offspring size within an individual female's clutch. As habitat variability decreases, the range should decrease because of consistent selection for an optimal phenotype.

Egg size also varies among clutches. A single female might produce clutches with very different mean egg sizes (Kaplan 1987). Kaplan and Cooper (1984) showed that in species that cannot "predict" the stability of the environment in which their larvae will grow and develop, the most efficient strategy will be to randomly produce a few large eggs or many small eggs. Interclutch variation in egg size in a female's lifetime (or within a population at one time) can outweigh the intraclutch variation of a female's single clutch.

Egg size has been shown to influence characters that relate to larval survival in salamanders (Kaplan 1980, 1985; Petranks 1984). Kaplan (1985) showed that in the newt *Taricha torosa* (Rathke, 1883) egg size can have profound effects on hatching size and growth rate. Thus, egg size might affect timing of metamorphosis. If so, then an optimal egg size can be selected for given a stable (or permanent) larval environment. In an unreliable environment, an optimal range of egg sizes might be the best strategy to maximize parental fitness. In *T. torosa*, large eggs produce large hatchlings that begin feeding sooner than smaller larvae. When fed ad libitum, larger larvae will metamorphose at an earlier time and at a larger size than conspecifics hatched from small eggs. In food-limited situations, larvae from larger eggs still metamorphose at a larger size but at a later time than larvae from small eggs. This interaction among egg size, food availability, and habitat reliability suggests that the optimal egg size can vary from season to season.

I present data on variation in egg size in five species of salamanders. Three questions are explored: (1) Does a species that uses temporary larval environments exhibit greater intraclutch variation in egg size than species that use more permanent larval habitats, such as mountain streams? (2) Is the total population variation in egg size greater in a species that uses temporary larval environments than in species that use mountain streams? (3) Is the

bulk of variation in egg size introduced at the intraclutch or interclutch level, and is this related to habitat variability?

MATERIALS AND METHODS

STUDY SPECIES

Ambystoma maculatum (Shaw, 1802) in the southern Appalachian Mountains usually breeds in mid-winter in temporary, seasonal pools that result primarily from heavy rains. Larvae emerge from eggs in early spring, and metamorphosis occurs 60-120 days later (Bishop 1941, Shoop 1974). The collection sites I used in this study dry completely 1-6 months after the rains, and on occasion they dry too early for any larvae to transform (R. C. Bruce, personal communication). Populations of *A. maculatum* in eastern North America are known to lay one mass of eggs per clutch (Wilbur 1977, Pfingsten and Downs 1989) or two or more masses per clutch (Bishop 1941, Pfingsten and Downs 1989). "Masses" will be referred to as "clusters" in this article. It is unknown if the clutch of a female *A. maculatum* at this locality consists of one or multiple clusters. Eggs of *A. maculatum* were collected in March 1988 from four temporary pools located in Blue Valley on the escarpment of the Blue Ridge Mountains, Macon County, North Carolina.

Eurycea wilderae Dunn, 1920, *Desmognathus santeelah* Tilley, 1981, and *D. ochrophaeus* Cope, 1859 lay eggs in and along headwater streams. These sites represent permanent sources of water, even during seasons of drought (W. Swank, Coweeta Hydrologic Laboratory, personal communication). Fishes are uncommon in these headwater streams. The permanence of these sources of water is evident when one considers that several species of plethodontids have larval periods in excess of 3 years, e.g., *D. quadramaculatus* (Holbrook, 1840) and *Gyrinophilus porphyriticus* (Green, 1827) (Bruce 1980, 1988a). *Eurycea wilderae* has a larval period of 1 or 2 years (Bruce 1988b). Females attach their eggs to the undersides of large rocks where the clutch is exposed to running water. *Eurycea wilderae* clutches were collected during February and March 1988 at Wolf Creek on Cullowhee Mountain, in the Cowee Mountains, Jackson County, North Carolina. Clutches were located by raking through cobble of headwater seepages.

Desmognathus ochrophaeus clutches were collected from various headwater streams in the Balsam Mountains in Haywood and Jackson counties, North Carolina. The Balsams are a southern extension of the Great Smoky Mountains, Swain County, North Carolina where the *D. santeelah* clutches were collected. *Desmognathus santeelah* and *D. ochrophaeus* females brood the eggs under moss on logs and

rocks in and along the edges of headwater streams and seepages. These species of *Desmognathus* have larval periods less than one year (Bruce 1989). Clutches of the *D. ochrophaeus* and *D. santeetlah* were collected during July and August 1987.

Desmognathus aeneus Brown and Bishop, 1947 females oviposit under moist logs and moss. This species is direct-developing (Wake 1966), and desiccation risk should represent less constraint to it. Clutches of *D. aeneus* were collected in the vicinity of Standing Indian Campground in the Nantahala Mountains, Macon County, North Carolina, in May 1988.

COLLECTION OF MATERIAL

As soon as I collected them, I placed egg clutches in individual plastic containers. If I found a brooding female with the clutch, I collected her and placed her with it. The plastic containers were placed in a cooler and returned to the laboratory where the egg clutches were assigned Harrison developmental stages (Duellman and Trueb 1986). I used a dissecting microscope equipped with an ocular micrometer to measure egg diameters to the nearest 0.1 mm. Late developmental stages were assigned based primarily on gill ontogeny. Embryos of clutches in which the embryos were in later stages of development were adjusted for developmental increases in size with the transformation formulas of Kaplan (1979). Clutches of *A. maculatum* and *D. aeneus* were all collected very early in development. The other three species were collected at various developmental stages, some of them at late stages. Embryo diameters for the plethodontid species in late development were recorded as the length of the longest axis of the embryo (Fig. 1). Using this measurement protocol, I observed that plethodontid embryos do not begin to increase in size until after Harrison stage 30 (Beachy 1988).

ANALYSES

Intraclutch variation was quantified by calculating the coefficient of variation (CV) for each clutch. The CV is a statistic that expresses the standard deviation as a percentage of the mean so that groups having very different means can be compared. These intraclutch CVs were subjected to a one-way ANOVA by species. Although a single female of *A. maculatum* may oviposit several clusters of eggs, I assumed that differences among these clusters do not contribute significantly to the variance in CVs, and all *A. maculatum* clusters were treated as though they were from different females (however, see Results).

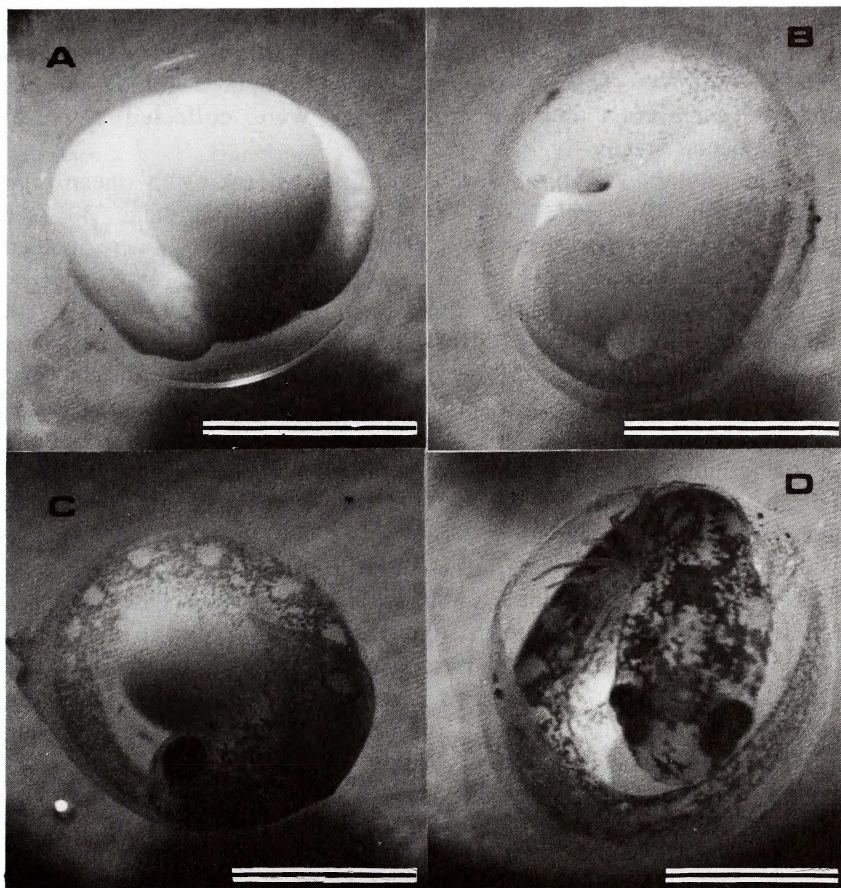


Fig. 1. *Desmognathus santeetlah* embryo at four different Harrison developmental stages: (A) stage 25, (B) stage 35, (C) stage 40, (D) stage 45. Note spherical orientation of embryo, even at late developmental stages. Bars = 2.0 mm.

Egg laying is not synchronous in plethodontid salamanders, and this precludes collection of a large number of plethodontid clutches at early stages of development. Therefore, I collected clutches at various stages of development. To ensure that intraclutch variation did not vary with development, a Wilcoxon's matched-pairs signed-ranks test was conducted on 12 *D. santeetlah* clutches to test if stage of development significantly influenced CV. Coefficients of variation were calculated for two developmental stages (stage 45 and an earlier stage, ranging from 9 to 41 for the clutches in question) for each clutch, and no significant difference was found in CV due

to development (\bar{x} early stage = 5.43, \bar{x} stage 45 = 5.96, $n = 12$, $T_s = 21$, ns). This result was assumed to hold true for *D. ochrophaeus* and *E. wilderae*. This assumption was not required for *D. aeneus* and *A. maculatum* because these eggs were collected at early developmental stages.

Interclutch variation was quantified by taking a mean egg diameter for all clutches at, or prior to, Harrison stage 30 (see above discussion on changes in size). These means were pooled according to species, and a CV was calculated for each species. These species CVs were squared and subjected to pairwise *F*-tests (Lewontin 1966). Kaplan (1987) showed that *Bombina orientalis* (Boulenger, 1890) females can produce clutches of different mean egg sizes. By assuming that this is the case for the species used in my study (i.e., the variation represented in a sample of the population might mirror the variation introduced by a single female in her lifetime), one can set predictions that are similar to those for intraclutch variation.

To determine the relative contributions of intraclutch and interclutch variation to the overall variance, egg size data for each species were subjected to a one-way ANOVA, with individual clutches as the factor. Relative contributions of intraclutch and interclutch variation to overall variance for each species were calculated using the factor and error sum-of-squares of the ANOVA table (Sokal and Rohlf 1981).

I analyzed data with StatView512+TM following the methods of Sokal and Rohlf (1981). In all analyses $\alpha = 0.05$.

RESULTS

My hypothesis was that the variation in egg size would be greatest in *A. maculatum*, the temporary pool breeder, and the lowest in *D. aeneus*, which is not constrained by habitat variability and thus should exhibit the greatest degree of canalization. Variation in egg size should be intermediate in the other three species. Descriptive statistics of egg size for the five species under study are presented in Table 1.

INTRACLUTCH VARIATION

Coefficients of variation of all clutches were analyzed with a model I one-way ANOVA (Sokal and Rohlf 1981), with species as treatment. Significant differences were found in intraclutch CV among species. A Fisher's PLSD a posteriori test was employed to determine the nature of the differences. The prediction that species using ephemeral larval habitats will display larger variation in egg size was not supported. Of all species, *D. aeneus* exhibited the

Table 1. Descriptive statistics of egg size for five species of salamanders.^a

Species	Number of clutches	Clutch size		Egg size (mm)	
		\bar{x}	SD	\bar{x}	SD
<i>A. maculatum</i>	37 ^b	67.24	34.71	3.15	0.40
<i>D. ochrophaeus</i>	6	15.00	3.56	3.32	0.11
<i>D. santeetlah</i>	29	20.01	6.00	3.43	0.25
<i>E. wilderae</i>	6	14.77	4.85	3.26	0.24
<i>D. aeneus</i>	10	11.80	2.23	2.54	0.13

^aData are calculated using mean egg size per clutch. Only data for those clutches at, or earlier than, Harrison developmental stage 30 are presented (see text for explanation).

^bRefers to clusters for *A. maculatum*.

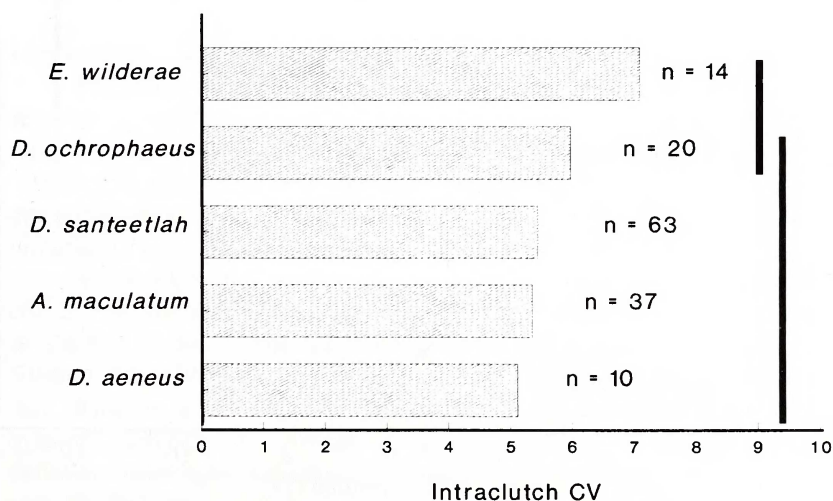


Fig. 2. Coefficients of variation (CV) of intraclutch variation in egg size for five species of salamanders. Number of clutches examined for each species is indicated. Vertical lines indicate means not significantly different using a model I ANOVA with species as treatment. ($\alpha = 0.05$).

lowest degree of variability, as expected. But *A. maculatum* did not exhibit the highest degree of intraclutch variation as was expected (Fig. 2).

INTERCLUTCH VARIATION

Because embryo diameter begins to increase in late developmental stages, only those clutches of *D. santeetlah*, *D. ochrophaeus*, and *E. wilderae* collected earlier than Harrison developmental stage 30 were used in this analysis. All egg size data for *A. maculatum* and *D. aeneus* were analyzed. Mean egg size was determined for each clutch, and those data were used to calculate a CV of egg size for each species (Table 1). In this analysis, *A. maculatum* did show the greatest variation in egg size (Fig. 3). Except for *E. wilderae*, all species CVs were significantly lower than that for *A. maculatum*. *D. aeneus* was predicted to exhibit the lowest degree of variation in egg size; only *D. ochrophaeus* has a lower CV, although this difference was not significant.

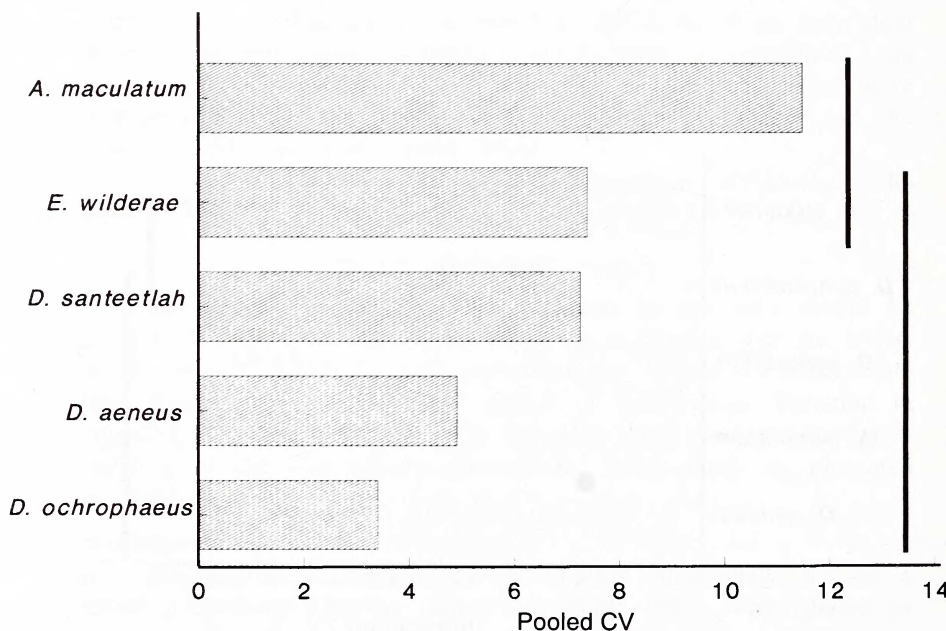


Fig. 3. Coefficients of variation (CV) of pooled (interclutch) variation in egg size for five species of salamanders. The species CVs are tested with an *F*-test of the squared CVs (Lewontin 1966). Vertical lines indicate means not distinguishable by pairwise *F*-tests ($P > 0.05$).

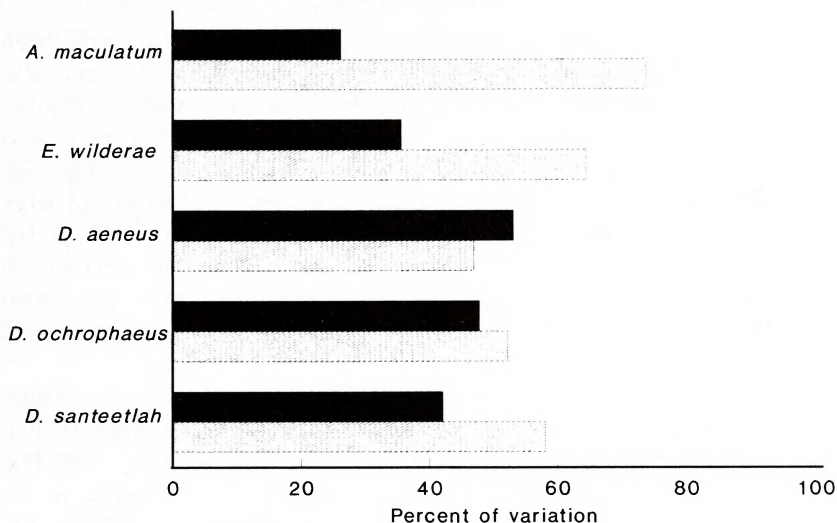


Fig. 4. Relative contributions of intraclutch and interclutch variation in egg size to overall variance. For each species, a separate model II one-way ANOVA of egg size was conducted with clutches as the factor, thus a total of five ANOVAs. Contributions are calculated using the factor and error sum-of-squares of the ANOVA table. Black bars represent intraclutch variation, and hatched bars represent interclutch variation.

INTERCLUTCH AND INTRACLUTCH CONTRIBUTION TO VARIATION

Partitioning of the variance components demonstrated the respective contributions of intraclutch and interclutch variation to the sample variance in egg size (Fig. 4). For *E. wilderae* and *A. maculatum*, interclutch contributions to the variance outweighed intraclutch contributions. In all three desmognathines, interclutch and intraclutch contributions were approximately equal.

As pointed out earlier, *A. maculatum* females may oviposit a clutch that consists of several clusters. The mean cluster size reported in Table 1 is lower than earlier reports of clutch size in *A. maculatum* (Bishop 1941, Wilbur 1977, Pfingsten and Downs 1989). This suggests that females of *A. maculatum* in the southern Appalachians may oviposit clutches that consist of several clusters. Because interclutch variation outweighs intraclutch variation in this species, the possibility remains that several clusters might contribute to a single *A. maculatum* clutch. This may confound the analysis of intraclutch variation, i.e., the intraclutch CVs for *A. maculatum* might be underestimates. However, the pooled variation remains as an indicator of potential adaptive variation in egg size at the interclutch level.

DISCUSSION

The potential role of variation in offspring size has long been a topic of debate. Kaplan and Cooper (1984) suggested that egg size variation in amphibians enables a female to produce viable offspring in unpredictable environments. In addition, Kaplan and Cooper (1984) submit that there are two levels at which variation in offspring size may be introduced: within a single clutch and among successive clutches of an individual female (i.e., at the intraclutch and interclutch levels). I tested these hypotheses by comparing the amount of variation in egg size observed in five species of salamanders that use larval environments ranging (in terms of safety from desiccation) from permanent to ephemeral.

These data add to the existing evidence showing that variation in egg size in amphibians is extensive. In addition, it appears that variation in offspring size differs among individual clutches and that the degree of variation differs among species. The question to be asked is whether the degree of variation is related to different life history strategies employed by species using different environments.

Kaplan and Cooper (1984) proposed that variation in amphibian egg size might reflect different reproductive strategies. In their attempt to model parental investment, they included a consideration of the extensive variation in propagule size seen in amphibians, insects, and plants. Earlier models of parental investment lacked this aspect (e.g., Smith and Fretwell 1974). A study by Crump (1981) emphasized the potential role of variation in amphibian egg sizes. Crump found no significant differences in egg size variation among species of treefrogs that use habitats of differing variability. However, among species that use temporary ponds, individual females produced clutches that tended toward a platykurtic distribution of egg size (bet hedging). Those species that breed in permanent ponds tended toward a leptokurtic distribution (canalization).

However, Crump's evidence has been criticized as unconvincing (McGinley et al. 1987). In a mathematical consideration of Smith and Fretwell's and Kaplan and Cooper's models, McGinley et al. (1987) suggested that variable environments do not necessarily select for variable parental investment in offspring. Parental fitness can be maximized, even in heterogeneous environments, by investing equally in all offspring. Is variation in egg size adaptive? Or are there factors that prevent a female from investing equally in all offspring?

If one were to consider only the intraclutch variation that I present here, the supposition that variation in egg size is correlated with desiccation risk appears to garner little support. The data on interclutch variation, however, suggest that species that use ephemeral

larval habitats might be able to introduce variation in egg size by ovipositing successive clutches with different mean egg sizes. *A. maculatum* did exhibit the largest degree of interclutch variation, and the other species followed in nearly the predicted order.

Smith-Gill (1983) suggested that much adaptive variation can be introduced at the whole organism level through developmental mechanisms, the mechanism in this case being vitellogenesis. Those developmental mechanisms that provide for variation should be subject to natural selection; i.e., those mechanisms should provide the amount of variation that maximizes an individual's fitness. The variation in egg size in the species examined provides some support for the hypothesis that this variation is correlated with habitat variability and is possibly adaptive. It remains to be seen if the hypothesis of adaptive variation in egg size is supported when more species of amphibians are investigated.

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